Original Research Papers

Solid Phase Extraction GC/ECD Method for the Analysis of Organochlorine Pesticides in Wildlife Plasma

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Summary

A gas chromatographic method for the analysis of nine organochlorine pesticides in wildlife plasma is described. Reversed-phase solid phase extraction is utilized to extract the organochlorine pesticides from plasma. This is followed by a normal phase solid phase extraction clean-up as the pesticides are recovered by elution with hexane:ethyl ether (1:1) and quantified by gas chromatography/electron capture detection. Method limits of detection range from 7.0–25 µg/L. The mean recovery for all pesticides is 81%.

1 Introduction

The Rocky Mountain Arsenal (RMA) Wildlife Refuge constitutes 6,900 hectares of short grass prairie located approximately 16 kilometers northwest of downtown Denver, CO. In addition to serving as a weapons production facility for the US Army, portions of the site had been leased by private corporations and used for the production of organochlorine pesticides. Such activities began in the late 1940's and were terminated by 1983 [1]. Waste disposal and storage practices typical for that time have contaminated areas of the refuge with a variety of chemicals. Numerous chemicals resulting from human activity have been identified on this Superfund National Priorities listed site [2].

Based on a number of factors including potential toxicological effects, chemical properties, environmental stability, and frequency of detection on the site, the top five chemicals of concern identified by both the U.S. Army and the United States Environmental Protection Agency are the organochlorine pesticides dieldrin, aldrin, endrin, DDT, and DDE. Due to the high frequency of detection in plants and animals sampled from the site, dieldrin has been determined to be the primary chemical of concern. To evaluate the effects of chemical contamination of the RMA Wildlife Refuge on wildlife, the U.S. Fish and Wildlife Service (USFWS) is conducting wildlife biomonitoring studies primarily for dieldrin. It is hoped that these studies will contribute to rational remediation strategies; optimally focusing resources on clean-up activities which will assure the safety of wildlife while preventing the unnecessary clean-up of areas which do not pose a significant threat.

Potential absorption of dieldrin by wildlife is typically via oral or dermal exposure. Oral LD_{50}s for mouse, rat, and guinea pig range from 38–49 mg/kg while dermal LD_{50}s range from 40–120 mg/kg [3]. As the oral and dermal LD_{50}s are quite similar, absorption of dieldrin is probably similar for both routes of exposure. Following either route of exposure, passive diffusion to blood accounts for substantial absorption [4]. While dieldrin's solubility in water is extremely low (186 µg/L) [3], dieldrin binds strongly to plasma proteins. In chronic dosing studies in which rats were fed 50 mg/kg of dieldrin over 6 months, blood dieldrin levels increased rapidly during the first two weeks but then plateaued at 240 µg/L for the remaining 26 weeks of the study [5]. Similar results were reported by Walker [6] for a study in which rats were fed 0.1–10 mg/kg/day for 104 weeks. In a chronic dosing study in which beagle dogs were dosed with 0.1 mg/kg/day for 128 days, dieldrin blood concentrations increased during the first 8 weeks and plateaued at 150 µg/L. In all chronic dosing studies, dieldrin partitioned to adipose until an equilibrium was reached, fluctuating minimally thereafter. Following a single 10 mg/kg oral dose of dieldrin to rats, blood concentrations peaked at 500 µg/L about 2 hours post dosing, decreased to about 200 µg/L during the next 48 hours, and then rapidly declined to 10 µg/L 8 days later [7]. As the dieldrin concentration in blood *vs* time mimicked dieldrin concentrations in fat, liver, kidney, brain, and muscle, blood dieldrin concentrations are a good indicator of the total body burden of dieldrin and hence exposure to dieldrin.

For the development of a biomonitoring procedure for dieldrin exposure which is adaptable to multiple wildlife species, the pharmacokinetics data indicate that the analysis of blood for dieldrin is promising. Furthermore, collection of blood from captured wildlife is relatively quick, non-lethal, and more humane than sampling other tissues such as adipose or muscle. Initially, to develop a model relating blood dieldrin levels with toxicity, captured wildlife will be dosed with dieldrin and blood plasma dieldrin levels determined over time. Relationships between plasma dieldrin levels and gross pathology, histopathology, various biochemical-physiological endpoints, residue levels, and dose levels will be determined. The development of an analytical method to quantify dieldrin and other organochlorine pesticides in wildlife blood/plasma is critical to the development of the model and implementation of this biomonitoring program. This paper reports the successful development and implementation of such an analytical method.

2 Materials and Methods

2.1 Equipment

A Hewlett-Packard Model 5890 Series II gas chromatograph equipped with electronic pressure control, dual electron capture detectors, and dual 7673A autosamplers was used to quantify organochlorine pesticides in plasma extracts. One gram, 6 mL, C18 (endcapped) solid phase extraction columns (SPE), one gram 6 mL silica SPE columns and Vacmaster(tm) sample processing stations were from Jones Chromatography, Lakewood, CO. Gas Chromatography (GC) expendables including inlet liners, silanized glass wool, and gold inlet seals were from Restek Corporation, Bellefonte, PA.

2.2 Chemicals

Neat organochlorine pesticide standards were obtained from Chem Service Inc., West Chester, PA. Ether, anhydrous 99+% was from Aldrich Chemical Co., Milwaukee, WI. Pesticide residue grade hexane was from Fisher Chemical, Fair Lawn, NJ. Bovine plasma was obtained from Sigma Chemical Co., St. Louis, MO.

2.3 Standard Preparation

Stock standards were prepared from neat materials and dissolved in acetone (1000 µg/mL) and diluted in acetone to prepare standard solutions for fortification (10 µg/mL). Instrument calibration stock (1000 µg/mL) and diluted (10 µg/mL) standard solutions were prepared in hexane.

2.4 Sample Fortification

Control bovine plasma was fortified with a mixed standard containing lindane, aldrin, heptachlor epoxide, *trans*-chlordane, *cis*-chlordane, *p'p'*-DDE, dieldrin, endrin, and *p'p'*-DDT. For method validation, control plasma was fortified at 5 levels: 25, 50, 100, 250, and 500 µg/L of each compound. For daily positive quality control samples, control plasma was fortified with each compound at 250 µg/L. Daily blank quality control samples and actual wildlife plasma samples were fortified with lindane only (surrogate standard) at 250 µg/L.

2.5 Sample Preparation

SPE columns (C18) were placed on the sample processing station and pre-conditioned with 3 × 6 mL aliquots of deionized water, the last aliquot eluted to the top of the column packing only. Frozen plasma samples were brought to room temperature and a 3 mL aliquot centrifuged for approximately 5 min at 4000 × g. A 1.0 mL aliquot of the centrifuged plasma was transferred to the SPE column using a Hamilton 1000 µL syringe. The sample was then fortified with the appropriate standard solution. After 1 hour equilibration at room temperature, the stopcock was opened and the plasma eluted through the C18 column at ambient pressure. When the elution had stopped, the remaining plasma was eluted through the column with a gentle vacuum (–50 mm Hg). The eluate was discarded and the column dried for 20 min under full vacuum (–500 mm Hg). After drying, the manifold needles were rinsed with hexane to remove any dried plasma, and the collection tubes removed and replaced with 10 ml tubes which had been calibrated to a 1.0 mL volume with hexane. To effect a cleaner final extract, a one gram 3 mL silica SPE column was placed in series below each C18 SPE column. The organochlorine pesticides were eluted with 4 × 3 mL aliquots of 1:1 hexane:ethyl ether, and the final amount of solvent in the column removed under gentle vacuum. The extracts were concentrated to less than 1.0 mL under a gentle stream of nitrogen in a fume hood, equilibrated to room temperature, and brought to a final volume of 1.0 mL with hexane. The samples were then capped, vortexed, and transferred to GC vials for pesticide quantification via gas chromatographic analysis.

2.6 Gas Chromatography

The gas chromatograph used a 250 °C inlet temperature and 350 °C detector temperature. The GC was PC controlled utilizing HP (Hewlett Packard) ChemStation software. The carrier gas was helium (25 cm/s) and the make-up gas was argon/methane (60 mL/min). The quantitation column was a 30 m × 0.25 mm i.d. fused silica, HP-5 crosslinked 5% phenyl methyl-silicone stationary phase, 0.25 µm film thickness (Hewlett-Packard). The confirmation column was a 30 m × 0.25 mm i.d. fused silica DB-17 bonded stationary phase, 0.15 µm film thickness (J&W Scientific).

The oven temperature program for quantitation and confirmation was as follows: 50 °C for 0.25 min, 60 °/min to 100 °C, 30 °/min to 190 °C hold for 2 min, 10 °/min to 300 °C hold for 5 min. The electronic pressure program for the quantitation column was 552 kPa for 2 min, 683 kPa/min to 110 kPa, held for 23 min. The confirmation column inlet pressure was held at a constant 110 kPa psi throughout the run. A 1.0 µL injection volume was used for the quantitation column with a single taper 4 mm i.d. inlet liner packed with deactivated glass wool. A 2 µL injection volume was used on the confirmation column which utilized a double taper 4 mm i.d. injection liner.

2.7 Method Validation

Detector linearity was determined by linear regression analyses of 5 point calibration curves (response *vs* mass analyte) for each analyte. After achieving $R^2 \geq 0.99$, linear regression equations were calculated and used to quantify analytes in samples. Fortified bovine plasma (5 levels) was cleaned up via the SPE procedure, analyzed by GC, and percent recoveries determined for each analyte at each fortification level on two consecutive days [8]. Method limits of detection (MLOD) were single point calculated from the chromatogram of the 25 µg/L fortified plasma. MLODs were calculated as the quantity of analyte required to give a response of 3 × baseline noise at the expected retention time of the analyte in the chromatogram of non-fortified plasma extract.

2.8 Quality Control

All wildlife plasma samples were fortified with 250 µg lindane/L during sample preparation. A positive control (fortified with all analytes at 250 µg/L) and blank control plasma sample (fortified with lindane at 250 µg/L) were also prepared and analyzed with each lot of 20 wildlife plasma samples.

To assure constant instrument performance, prior to the GC analysis of any samples, endrin and DDT degradation was ≤ 20% for each compound and ≤ 30% for both compounds as indicated by the analysis of a 250 µg/L endrin and DDT standard. Also, a 250 µg/L instrument calibration check standard was analyzed at the beginning, after every ten samples, and at the end of each analytical run. The response for the majority (2/3) of the analytes and all compounds positively identified in the samples was required to match the response of the 250 µg/L standard in the calibration curve ± 25%.

Method performance was monitored and documented by tracking recoveries of five of the fortified analytes; aldrin, dieldrin, endrin, *p'p'*-DDT and *trans*-chlordane. Surrogate recoveries were used to monitor individual sample extraction proficiency and instrument performance. In addition to analysis on the quantitation column, extracts of all plasma samples found to contain organochlorine pesticides were confirmed by GC analysis on the confirmation column.

3 Results and Discussion

3.1 Chromatography

Due to the commercial unavailability of wildlife plasma, bovine plasma was utilized for method development, validation, and quality control samples. Control bovine plasma proved to be acceptable for these purposes as indicated by the chromatograms presented in **Figure 1**. The chromatograms from the analyses of bovine plasma and plasma collected from a Great Horned Owl trapped at an uncontaminated site were quite similar. Both chromatograms are free of interfering peaks at the retention times of the organochlorine pesticides of interest as indicated by the chromatogram of fortified bovine plasma.

3.2 Method Validation

The results of the method validation experiments are presented in **Table 1**. For dieldrin, the analyte of primary concern, the mean recovery was 82.6% and the standard deviation was 10.3%. Mean

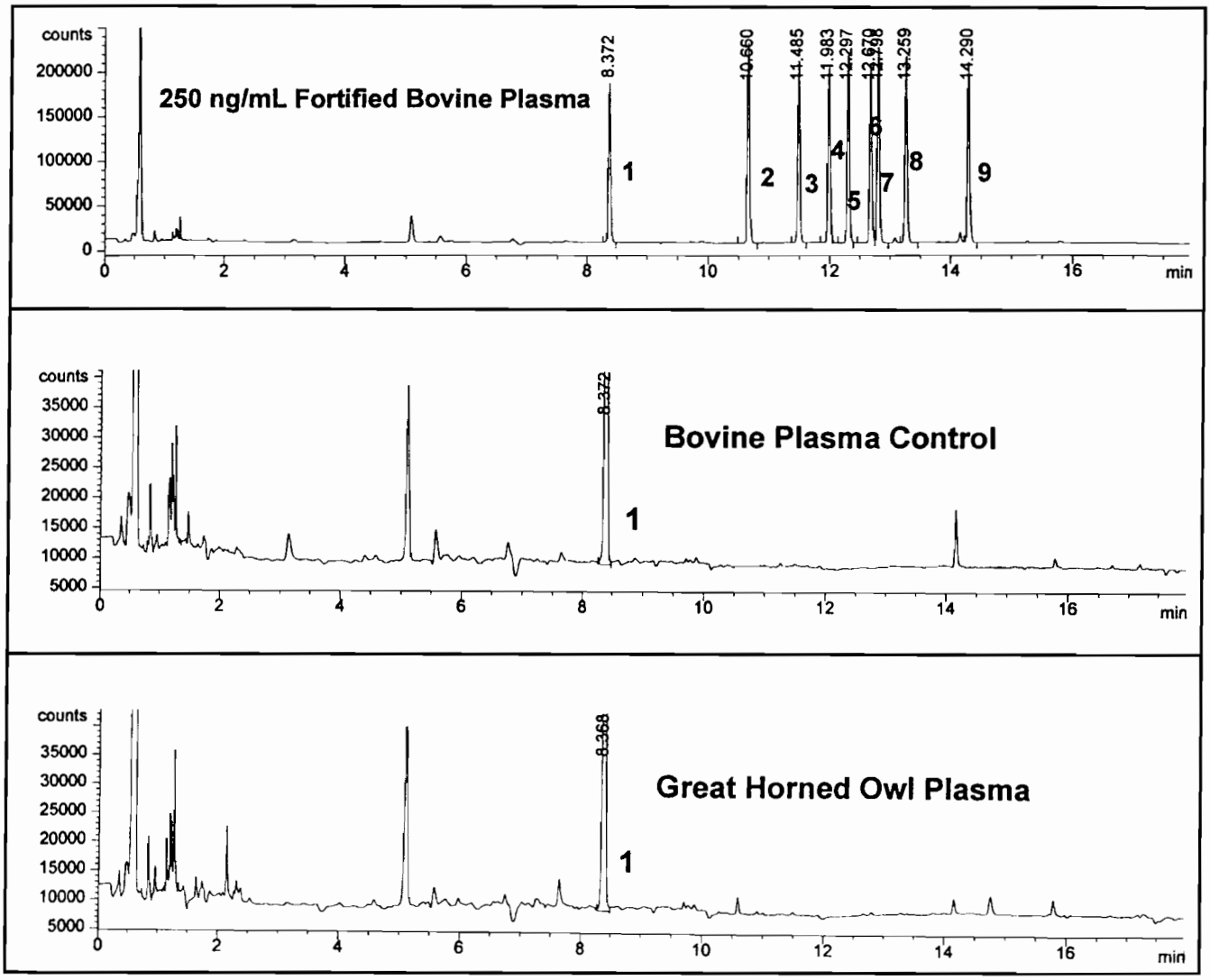

Figure 1. Chromatograms of 250 µg/L fortified bovine plasma, control bovine plasma and great horned owl plasma. **(1)** lindane (surrogate), **(2)** aldrin, **(3)** heptachlor epoxide, **(4)** *trans*-chlordane, **(5)** *cis*-chlordane, **(6)** *p,p'*-DDE, **(7)** dieldrin, **(8)** endrin, and **(9)** *p,p'*-DDT.

Table 1. Method validation mean percent recovery data.

Compound	Fortification level µg/L 25	50	100	250	500	Overall mean	Standard dev.
Lindane	77	85	65	85	82	78.8	8.4
Aldrin	73	82	60	79	76	74.0	8.5
Heptachlor epox.	80	91	71	97	92	86.2	10.5
trans-Chlordane	73	83	63	87	81	77.4	9.5
cis-Chlordane	76	87	66	89	82	78.7	9.0
p,p'-DDE	66	79	56	76	68	69.0	9.1
Dieldrin	76	89	68	93	87	82.6	10.3
Endrin	80	95	74	101	92	88.4	11.1
p,p'-DDT	80	100	78	104	96	91.6	11.9

Table 2. Method limits of detection in plasma.

Compound	Limit of detection µg/L
Lindane	25
Aldrin	13
Heptachlor epoxide	15
trans-Chlordane	15
cis-Chlordane	14
p,p'-DDE	7.0
Dieldrin	7.0
Endrin	7.0
p,p'-DDT	7.0

recoveries of the other analytes of primary concern, aldrin, endrin, DDT, and DDE were 74.0 ± 8.5%, 88.4 ± 11.1%, 91.6 ± 11.9%, 69.0 ± 9.1%, respectively. Mean recoveries of lindane, the compound added to all samples as a surrogate standard, was 78.8 ± 8.4%. This is similar to the 80.7 ± 7.2% mean recovery for all compounds, indicating the suitability of lindane as a surrogate standard for these analyses. MLODs are presented in **Table 2**. The MLOD for dieldrin was 7.0 µg/L. The MLODs for the other analytes of primary concern ranged from 7.0 µg/L for endrin, DDT, and DDE to 15 µg/L for *trans*-chlordane and heptachlor epoxide.

Table 3. Recovery data of surrogate and fortified analytes in plasma.

Compound	Percent recovery	Standard deviation
Lindane [a]	90	10.9
Aldrin [b]	64	17.5
Heptachlor epoxide [b]	92	6.7
trans-Chlordane [b]	68	4.5
cis-Chlordane [b]	69	4.8
p,p'-DDE [b]	59	4.9
Dieldrin [b]	77	5.7
Endrin [b]	90	8.2
p,p'-DDT [b]	78	6.9

[a] Lindane surrogate recovery is grand mean of 300 analyses in actual plasma samples.
[b] Mean recovery of 17 fortified bovine plasma samples analyzed concurrently with wildlife plasma samples.

3.3 Analysis of Wildlife Plasma

As indicated by the recovery data presented in **Table 3**, this method proved sufficiently rugged for the analyses of plasma samples collected from animals on the RMA. During the analyses of 340 samples, recoveries of the lindane surrogate were quite consistent as indicated by the average recovery ± standard deviation of 90 ± 10.9%. Recoveries of dieldrin from the fortified control plasma analyzed with each lot of 20 samples averaged 77 ± 5.7%. Dieldrin was detected in plasma collected from badgers (*Taxidea taxus*) which had been trapped in uncontaminated areas and dosed with dieldrin and from badgers trapped on the RMA. These data will be used to generate the pharmacokinetics model. Plasma collected from a variety of other animals trapped on the RMA have been analyzed by this method. These animals included great horned owls (*Bubo virginianus*), kesteral falcons (*Falco sparverius*), Swainson's hawks (*Buted swainsoni*), bald eagles (*Haliaeetus leucosephalus*), golden eagles (*Aquila chrysaetos*), magpies (*Pica pica*), and starlings (*Sturnus vulgaris*). A chromatogram from the analyses of plasma from blood collected from a Great Horned Owl trapped on the RMA is shown in **Figure 2**. The presence of dieldrin is indicated by the peak at retention time of 12.796 min and was confirmed by GC analysis on the confirmation column.

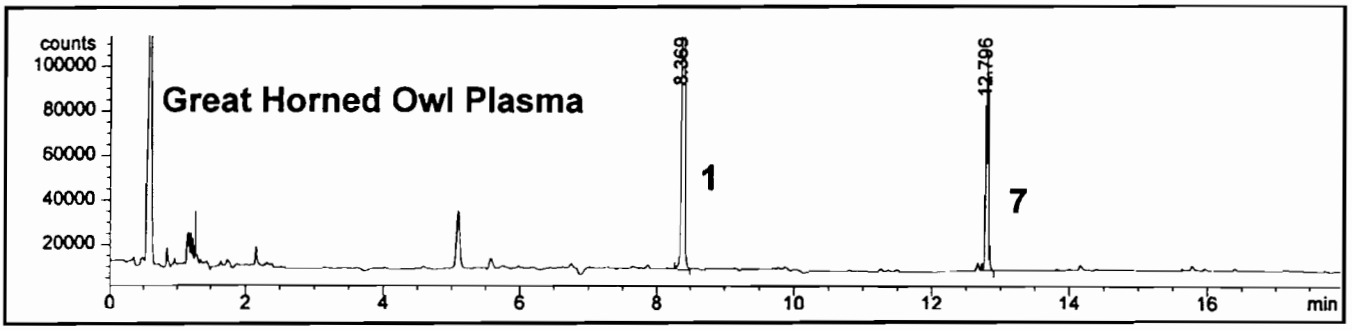

Figure 2. Chromatogram of great horned owl plasma sample containing lindane surrogate (**1**) and incurred dieldrin residue (**7**).

Using multiple sample processing stations, an analyst typically prepared 40 samples and 4 quality control samples for GC analyses in 4 hours. These 40 samples were usually analyzed by GC in 2 lots of 20, each lot analyzed on separate days. Each sample required 6 mL of ether and 8 mL of hexane, with < 1 mL of hexane remaining for disposal after analysis.

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Reversed Phase HPLC Coupled On-Line to GC by the Vaporizer/Precolumn Solvent Split/Gas Discharge Interface; Analysis of Phthalates in Water

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Summary

Reversed phase liquid chromatography (RPLC) was coupled on-line to gas chromatography (GC) via the vaporizer chamber/precolumn solvent split/gas discharge interface outlined recently. Water-containing eluents were driven into a vaporizer chamber at 300 °C by the LC pump. The vapors were removed through an early vapor exit by the carrier gas. Solvent/solute separation occurred in the retaining precolumn. Special attention was paid to the parameters determining the losses of the most volatile compounds. The oven temperature during transfer was lowered close to the dew point of the eluent (the temperature at which recondensation occurs) in order to maximize the retention power of the retaining precolumn. The dew point depends on the transfer rate, the gas flow rate, and the gas inlet pressure. Sometimes even better retention of the volatiles was observed at temperatures below the dew point, *i.e.* despite partial recondensation. The method was applied to the analysis of phthalates in drinking and surface waters. The detection limits, using MS, were 5–10 ng/l.

1 Introduction

On-line coupling of reversed phase HPLC (RPLC) to GC now looks back on a history of more than 10 years and has been described in references [2–8]. Ideas went through phase switching in LC, on-line liquid/liquid extraction with subsequent phase separation, extraction of the eluent into the stationary phase of a GC capillary precolumn, concurrent eluent evaporation with the loop-type interface, concurrent evaporation with co-solvent trapping, transfer through a PTV injector, and solid phase extraction/thermal desorption. According to our knowledge, however, none of these concepts has been turned into a widely used routine method.

In the past, numerous, fundamentally different techniques have been studied for on-line transfer of LC fractions [9,10] as well as for injection of large volumes. While this was important for exploring the possibilities, it is confusing for inexperienced users. In the near future, the most promising technique should be selected with the aim of combining the best concepts presented so far. It may include various options and variations, but it should follow a single basic idea that is easy to communicate. In particular, large volume injection and LC-GC transfer techniques should be unified. The RPLC-GC technique described here fits into a concept suitable for all these techniques.

This paper describes LC-GC transfer by the vaporizer/precolumn solvent split/gas discharge system [11], comprising the following steps: The eluent is vaporized in a chamber kept at a high temperature (typically 250–350 °C). Such high temperatures are needed to ensure sufficient heat supply to the evaporating liquid: it must be avoided that the evaporation process cools the chamber down to the boiling point of the sample (eluent). As shown in ref. [12], a chamber at 300 °C permits evaporation of water at a rate of up to around 200 µl/min – at higher introduction rates, unevaporated liquid flowed through the chamber into the (pre)column.

Solvent evaporation in a chamber kept far above the boiling point may be violent. Smooth evaporation requires immediate contact of the introduced liquid with a packing material, *i.e.* the transfer line must end on the packing material. Since the transfer line should not enter far into the hot zone (to avoid evaporation inside this line), the packing material was situated in the top part of the properly heated zone.

The vaporizing chamber consisted of a 1 mm i.d. glass tube, packed with a 2 cm plug of Carbofrit and internally coated with polyimide [13]. Polyimide behaves as a solid, *i.e.* exhibits no significant retention power. It is well suited for gluing the packing material to the liner wall and securing loose particles. At the same time, the wall of the glass tube is protected against attack by water.

Solvent vapors were largely discharged through an early vapor exit, driven by the flow of carrier gas ("gas discharge", in contrast to "overflow" [14]). Solvent/solute separation occurred in a coated capillary precolumn (retaining precolumn) upstream of this exit. Since water and water/methanol mixtures do not wet precolumn surfaces, it is impossible to apply solvent trapping to improve the retention of the volatile solutes. Thus, there was no purpose in installing an uncoated precolumn.

This transfer technique was applied to the LC-GC analysis of phthalates in drinking and surface water. LC served for enrichment of the phthalates, as well as for preseparation, *i.e.* for isolation from other materials extracted from the water. Phthalates are widespread in the environment and are of concern because they are considered carcinogenic. Their wide range of molecular weights also makes them an ideal training set for the observation of losses of the most volatile components.